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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	761075
Supporting document/s:	1
Applicant's letter date:	December 9, 2016
CDER stamp date:	December 9, 2016
Product:	FULPHILA (MYL-1401H Solution for Subcutaneous Injection)
Indication:	Being developed for the same indication without exclusivity as approved for the reference product US-licensed Neulasta
Applicant:	Mylan GmbH
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Natalie E Simpson, PhD
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1 Executive Summary

1.1 Introduction

Mylan GmbH is requesting marketing approval for MYL-1401H solution for subcutaneous (SC) injection (FULPHILA), as a proposed biosimilar product to the pegfilgrastim reference product, US-licensed Neulasta (also referred in this review as US-Neulasta). US-Neulasta was approved in 2002 (NDA 125031) and the current label includes indications and usage information for treatment of: (1) decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia (the proposed indication for MYL-1401H); and, (2) to increase survival in patients acutely exposed to myelosuppressive doses of radiation (Hematopoietic Subsyndrome of Acute Radiation Syndrome). The latter indication was granted approval under an orphan designation, with exclusivity until November 13, 2022.

Pegfilgrastim is a PEGylated granulocyte colony-stimulating factor that binds to granulocyte colony stimulating factor receptor (GCSF-R) on the surface of hematopoietic cells. The activation of the GCSF-R leads to subsequent phosphorylation and activation of the non-receptor tyrosine kinases JAK1 and JAK2 and induction of various neutrophil specific genes, thereby controlling precursor cell differentiation to neutrophils, neutrophil proliferation, and release of mature neutrophils from the bone marrow.[1,2,3]

As part of Mylan's developmental strategy for MYL-1401H solution for SC injection, MYL-1401H was compared head-to-head with European Union-approved Neulasta (EU-Neulasta) in a Sprague-Dawley rat study assessing pharmacodynamics (PD), toxicity, and toxicokinetics (TK). MYL-1401H was also compared head-to-head with EU- and US-Neulasta in a PD study in neutropenic rats to bridge the three pegfilgrastim products. The rat is an appropriate species for the comparative animal studies because pegfilgrastim is known to be pharmacologically active in rats. The nonclinical development of MYL-1401H involved selecting doses, a route of administration, and a dosing regimen consistent with the currently approved labeling of US-Neulasta. The totality of the nonclinical data indicates that MYL-1401H is similar to US-Neulasta.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical data submitted to the BLA demonstrate similar pharmacodynamic responses of MYL-1401H, US-Neulasta, and EU-Neulasta. The PD similarity provides

[1] Nicholson SE, Oates AC, Harpur AG, Ziemiecki A, Wi ks AF, Layton JE, 1994, Tyrosine kinase JAK1 is associated with the granulocyte-colony-stimulating factor receptor and both become tyrosine-phosphorylated after receptor activation, Proc Natl Acad Sci, 91(8), 2985-2988.
[2] Shimoda K, Iwasaki H, Okamura S, Ohno Y, Kubota A, Arima F, et al, 1994, G-CSF induces tyrosine phosphorylation of the JAK2 protein in the human myeloid G-CSF responsive and proliferative cells, but not in mature neutrophils, Biochem Biophys Res Commun, 203(2), 922-8.
[3] Fukunaga R, Ishizaka-Ikeda E, Nagata S, 1993, Growth and differentiation signals mediated by two distinct regions in the cytoplasmic domain of granulocyte colony-stimulating factor receptor, Cell, 74(6),1079-1087.

a bridge to support a demonstration of biosimilarity for toxicity and TK endpoints between MYL-1401H and EU-Neulasta to US-Neulasta.

Two GLP-compliant comparative studies were submitted to support the similarity of MYL-1401H and US-Neulasta: a 3-way comparative single dose PD study in male neutropenic rats with MYL-1401H, EU- and US-Neulasta and a 2-way comparative PD, toxicity, and TK study in Sprague-Dawley rats with MYL-1401H and EU-Neulasta. In the single dose PD study, SC doses of MYL-1401H, US-Neulasta, and EU-Neulasta were administered at doses of 100, 300, 1000, and 3000 µg/kg in a chemically induced (cyclophosphamide-treated) rat model. No local intolerance, changes in body weight, changes in food consumption, or mortalities were observed. There were no statistically significant differences (indicated by $p \leq 0.05$, Student's t-test) in induced leucocyte and neutrophilic granulocyte levels (measured by absolute neutrophil count (ANC)) among the pegfilgrastim products at the doses tested. The ratios of the test and reference items based on the area under the effect-time curve ($AUEC_{eff}$) for ANCs were compared pairwise between treatment groups, and were near unity for MYL-1401H versus (vs) US-Neulasta (0.952 to 1.269), MYL-1401H vs EU-Neulasta (1.201 to 1.497), and US-Neulasta vs EU-Neulasta (0.950 to 1.262). Other changes in hematology parameters following MYL-1401H, US-Neulasta or EU-Neulasta treatment displayed similar trends.

In the 2-way comparative toxicology study in male and female rats, MYL-1401H and EU-Neulasta were administered to Sprague-Dawley rats via the SC route at doses of 0 (vehicle control), 0.15, 0.65, or 1.5 mg/kg MYL-1401H, or 0.15 or 1.5 mg/kg EU-Neulasta once per week for 28 days (5 dosing occasions). One animal in the 1.5 mg/kg EU-Neulasta group was sacrificed moribund on Day 28 with clinical signs associated with granulocytic leukemia. MYL-1401H and EU-Neulasta treatment overall resulted in similar toxicities. Decreased red blood cell (RBC) parameters (total RBCs, hemoglobin, hematocrit) and platelets with increased spleen weight and size were observed for MYL-1401H and EU-Neulasta. Dose-related increases in leukocytes and ANC were observed with both MYL-1401H and EU-Neulasta. Alkaline phosphatase (ALP) levels were increased with an increase in dose for both MYL-1401H- and EU-Neulasta-treated rats when compared to controls. Additionally, increased aspartate aminotransferase (AST) levels were observed in 0.65 mg/kg/day MYL-1401H and 0.15 or 1.5 mg/kg/day EU-Neulasta groups (males only). Also observed were similar microscopic findings of increased granulopoiesis in the bone marrow (femur and sternum) as well as increased hematopoiesis in the spleen and liver. At the injection sites, minimal fasciitis/fibrosis and occasional hemorrhage were recorded in rats given MYL-1401H or EU-Neulasta and in control rats. Upon evaluation of TK parameters, similar greater than dose-proportional increases in mean maximal concentration (i.e., C_{max}), systemic exposure (i.e., AUC), and half-lives were seen with MYL-1401H and EU-Neulasta at 1.5 mg/kg, but C_{max} and AUC values for MYL-1401H were lower at the 0.15 mg/kg dose compared to EU-Neulasta.

The totality of the nonclinical data indicates that MYL-1401H is similar to US-Neulasta. There are no discipline-specific uncertainties regarding the overall similarity of MYL-1401H to US-Neulasta from the Pharmacology/Toxicology perspective.

1.3 Recommendations

1.3.1 Approvability

From the Pharmacology/Toxicology perspective FULPHILA may be approved for the proposed indication without exclusivity.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The nonclinical sections of the label will reflect data from the label of the reference product US-Neulasta, with minor changes to update for the Pregnancy and Lactation Labeling Rule (PLLR).

2 Drug Information

2.1 Drug

CAS Registry Number	208265-92-3
Code Name	MYL-1401H; pegylated granulocyte colony stimulating factor (PEG-GCSF); (b) (4) biosimilar
Chemical Name	N-(3-hydroxypropyl) methionyl colony-stimulating factor (human), 1-ether with α-methyl-ω-hydroxypoly (oxyethylene)
Molecular Formula/ Molecular Weight	$C_{845}H_{1339}N_{229}O_{223}S_9$ / 39 kDa
Structure or Biochemical Description	1 MTPLGPASSL PQSFLLKCLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL 51 LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH SGLFLYQGLL QALEGISPEL 101 GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG 151 GVLVASHLQS FLEVSYRVLR HLAQP
Pharmacologic class	Recombinant granulocyte colony stimulating factor (G-CSF)

2.2 Relevant INDs, NDAs, BLAs and DMFs

Pre-IND 123389; NDA 125031 (US-licensed Neulasta)

2.3 Drug Formulation

The MYL-1401H drug product (DP) is a clear, colorless, preservative-free, sterile solution for subcutaneous injection. It is supplied in a single-use prefilled syringe (PFS) containing 0.6 mL of the solution at a protein concentration of (b) (4) and at a pH of 4.0 (b) (4) Each PFS contains 6 mg of drug product. The PFS is presented with a passive needle guard (b) (4) UltraSafe™ Plus to reduce the occurrence of needle-stick injuries post injection. The DP is supplied as a 6 mg solution for injection (for subcutaneous use). Therefore, no diluent is required for reconstitution. The qualitative composition of the DP is formulated to be the same as that of the reference product, US-Neulasta.

Table 1 Composition of MYL-1401H Drug Product

(Excerpted from Module 3.2.P.1.)

Component	Function	Quantity per PFS	Reference to Standards
MYL-1401H	Active ingredient	6 mg	In- House
D-Sorbitol	(b) (4)	30 mg	Ph. Eur./USP
Polysorbate 20		0.024 mg	Ph. Eur./USP
Acetate[a]		0.7 mg	Ph. Eur./USP
Sodium[a]		0.01 mg	Ph. Eur./USP
Water for injection		q.s to 0.6 mL	Ph. Eur./USP

Abbrevations: Ph. Eur. = European Pharmacopeia; q.s.=quantity sufficient; USP = United States Pharmacopeia

(b) (4)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Mylan proposes the currently approved Neulasta indication without exclusivity (decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia) and dosing regimen consistent with current US-Neulasta labeling. See the approved US-Neulasta label for more detailed information.

2.7 Regulatory Background

BLA 761075 was submitted on December 9, 2016 for the biologic product MYL-1401H under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k). To fulfill the nonclinical requirements for a biosimilar BLA, the Applicant submitted a comparative PD study with a side-by-side comparison of MYL-1401H to US-Neulasta and EU-Neulasta (bridging MYL-1401H, US- and EU-Neulasta) and a 4-week comparative toxicology study that included PK, PD, and toxicity assessments comparing MYL-1401H side-by-side to EU-Neulasta. A pre-IND meeting to discuss the sufficiency of nonclinical data to support the clinical program was held on February 17, 2015 (teleconference), but the animal studies were not submitted for review prior to BLA submission. An Application Orientation Meeting was not held.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study No.	Title	Module
PHA 071-001	Pharmacodynamic Study of Pegfilgrastim (Mylan) Compared to EU-Neulasta and US-Neulasta Following Single Subcutaneous Administration to	4.2.1.1.

	Neutropenic Rats	

Repeat-Dose Toxicology

Study No.	Title	Module
TOX-071-001	Comparative 28 Day Subcutaneous Repeat Dose Toxicity Study in Sprague Dawley Rats of a Proposed Biosimilar Pegylated Recombinant Human Granulocyte Colony-stimulating Factor (Peg-GCSF) and European Sourced Neulasta Followed by a 2 Week Treatment-free Recovery Period.	4.2.3.2.

3.2 Studies Not Reviewed

Pharmacokinetics

Study No.	Title	Module
30240	Validation of an ELISA Method for the Quantification of US-Neulasta in Placebo Buffer	4.2.2.1.
8287639	Validation of a Method for Quantification of Neulasta and [redacted] (b) (4) in Rat Plasma (EDTA K2) using an ELISA	4.2.2.1.
8288957	Validation of a Method for Quantification of Neulasta and [redacted] (b) (4) Formulation Samples using Enzyme-Linked Immunosorbent Assay (ELISA)	4.2.2.1.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Study title: Pharmacodynamic Study of Pegfilgrastim (Mylan) Compared to EU-Neulasta and US-Neulasta Following Single Subcutaneous Administration to Neutropenic Rats

Study no:	PHA 071-001
Study report location:	eCTD 4.2.1.1.
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 11, 2013
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	Biosimilar ([redacted] (b) (4)), Batch No. S13DBPEGI-0005, Purity: 98.1%; US-Neulasta (10 mg/mL), Batch No. 1036958, Purity: not noted; EU-Neulasta (9.8 mg/mL), Batch No. 1037627, Purity: not noted, but assumed to be 97.3% since it is the same batch from Study No. TOX-071-001.

Key study findings:

- There were statistically significant differences in the number of leukocytes and neutrophilic granulocytes between cyclophosphamide-treated and untreated control rats, indicative of a valid chemically induced neutropenic assay to compare the pharmacodynamic effects of pegfilgrastim products.

- There were no test item-related statistically significant differences in the number of leukocytes or neutrophilic granulocytes between the biosimilar and US- or EU-Neulasta, or between US- and EU-Neulasta following a single subcutaneous administration.

Methods

Doses: (excerpted from the study report)

Group 1:	Control 1 (vehicle)
Group 2:	Control 2 (50 mg CPA/kg b.w. + vehicle)
Group 3:	100 µg US Neulasta®/kg b.w.
Group 4:	300 µg US Neulasta®/kg b.w.
Group 5:	1000 µg US Neulasta®/kg b.w.
Group 6:	3000 µg US Neulasta®/kg b.w.
Group 7:	100 µg EU Neulasta®/kg b.w.
Group 8:	300 µg EU Neulasta®/kg b.w.
Group 9:	1000 µg EU Neulasta®/kg b.w.
Group 10:	3000 µg EU Neulasta®/kg b.w.
Group 11:	100 µg Biosimilar/kg b.w.
Group 12:	300 µg Biosimilar/kg b.w.
Group 13:	1000 µg Biosimilar/kg b.w.
Group 14:	3000 µg Biosimilar/kg b.w.

Frequency of dosing:	Single dose
Route of administration:	Subcutaneous (SC)
Dose volume:	1 mL/kg (20 mL/kg for CPA)
Formulation/Vehicle:	(b) (4) Polysorbate-20 (w/v), water for injection (placebo for PEG-G-CSF injection)
Species/Strain:	Rat/CD®/Crl:CD(SD) (males)
Number/Group:	10/group
Age:	61 days old (at dosing)
Weight:	300.2 to 372.4 g (at dosing)
Unique study design:	Neutropenia was chemotherapy-induced with a single intraperitoneal dose of 50 mg cyclophosphamide (CPA)/kg b.w. (body weight) one day before actual dosing with the test items or the vehicle (24-hour gap between CPA injection and pegfilgrastim or vehicle injection).
Deviation from study protocol:	There were no deviations determined by the Study Director to affect the integrity of the study or interpretation of results.

Results

Blood sampling days: pretest (Days -7, -2), daily from Day 1 until Day 8, and on Day 12.

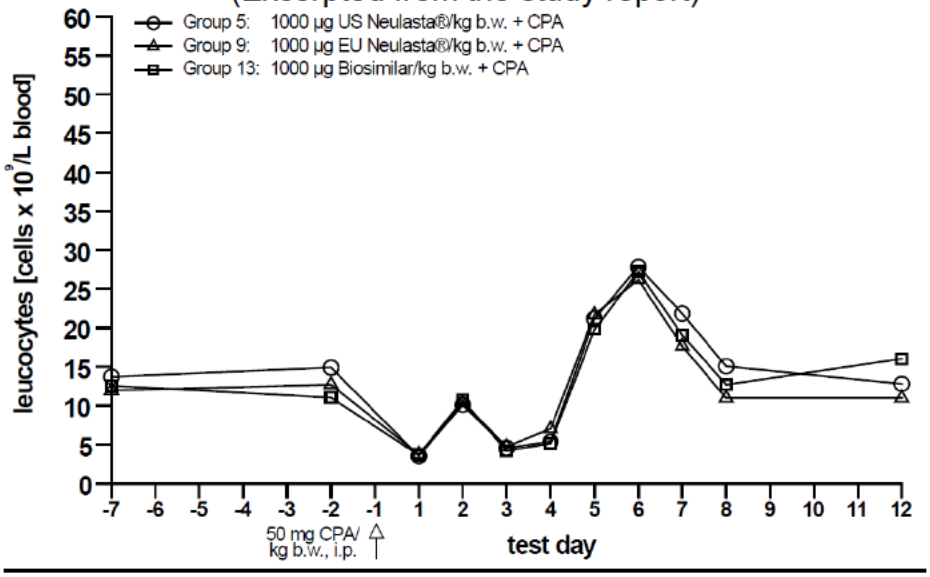
No deaths were noted. No local intolerance reactions were noted at the injection sites. No test item-related changes in behavior, external appearance or feces as well as for

body weight and food and water consumption were noted for the controls or the test item-treated animals.

Pharmacodynamic Leukocyte Response

Figure 1 Side-by-Side Comparison of US- and EU-Neulasta and the Biosimilar in Leukocyte Response at 1000 µg/kg

(Excerpted from the study report)



Note: only the 1000 µg/kg dose is highlighted in this figure since it covers the rat dose, based on body surface area (~600 µg/kg), equivalent to the human therapeutic dose to be administered to patients (6 mg, or 100 µg/kg assuming a 60 kg adult)

Table 2 Pharmacodynamic Analysis of Leukocyte Response

(Adapted from the Applicant's table in the study report)

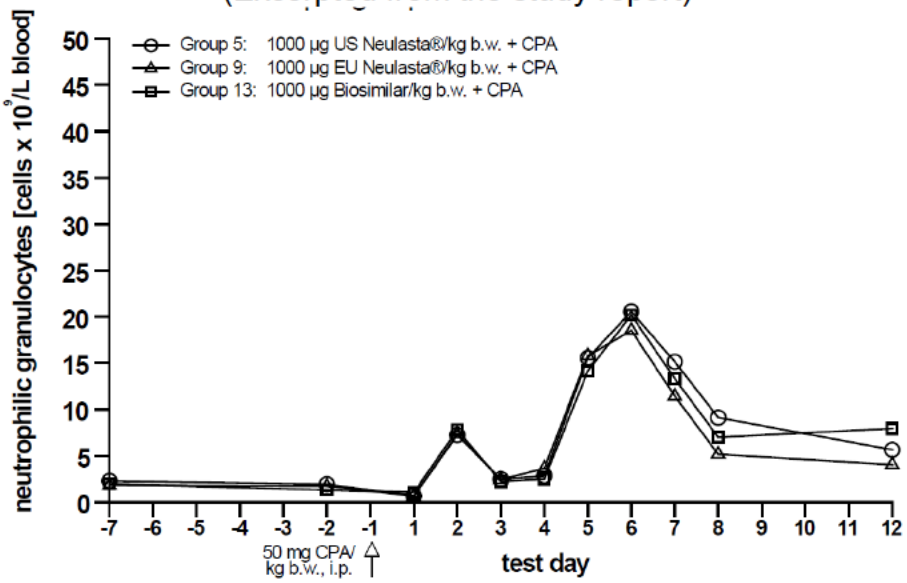
Group No.	Group Name	Dose [µg/kg]	E_{max} [cells x 10^9/L]	t_{max} [hr]	$AUEC_{0\text{-t last}}$ [hr*cells x 10^9/L]	$AUEC_{eff\ 0\text{-t last}}$ [hr*cells x 10^9/L]#
1	Control	0	15.01	110	3451.68	-
2	Control+CPA	0	10.05	264	1355.4	-
11	Biosimilar	100	15.12	113	1962.84	607.44
3	US-Neulasta	100	11.88	86	2057.76	702.36
7	EU-Neulasta	100	11.04	96	1815.36	459.96
12	Biosimilar	300	12.77	86	2009.4	654
4	US-Neulasta	300	12.45	118	2042.88	687.48
8	EU-Neulasta	300	11.13	120	1843.2	487.8
13	Biosimilar	1000	28.61	134	3640.08	2284.68
5	US-Neulasta	1000	30.35	118	3741.6	2386.2
9	EU-Neulasta	1000	27.05	115	3334.32	1978.92
14	Biosimilar	3000	59.82	127	5961.84	4606.44
6	US-Neulasta	3000	48.82	122	5005.08	3649.68
10	EU-Neulasta	3000	51.53	137	5235	3879.6

Note: Groups 3-14 were all pretreated with CPA and $AUEC_{0\text{-t last}}$ was calculated using the linear trapezoidal rule with leukocyte numbers representing "plasma concentrations" collected between time zero (Day 1) and tlast (Day 12).
#: effective AUEC of the dose level groups 3 to 14: $AUEC_{Gr.\ 3\ to\ 14} - AUEC_{Gr\ 2}$; E_{max} = maximum response; t_{max} = time of E_{max}; AUEC = area under effect-time curve.

Pharmacodynamic Neutrophilic Granulocyte Response

Figure 2 Side-by-Side Comparison of US- and EU-Neulasta and the Biosimilar in Neutrophilic Granulocyte Response at 1000 µg/kg

(Excerpted from the study report)



Note: only the 1000 µg/kg dose is highlighted in this figure since it covers the rat dose, based on body surface area (~600 µg/kg), equivalent to the human therapeutic dose to be administered to patients (6 mg, or 100 µg/kg assuming a 60 kg adult)

Table 3 Pharmacodynamic Analysis of Neutrophilic Granulocyte Response

(Adapted from the Applicant's table in the study report)

Group No.	Group Name	Dose [µg/kg]	E_{max} [cells x 10^9/L]	t_{max} [hr]	$AUEC_{0\text{-}t\ last}$ [hr*cells x 10^9/L]	$AUEC_{eff\ 0\text{-}t\ last}$ [hr*cells x 10^9/L]#
1	Control	0	2.598	103	483.84	-
2	Control+CPA	0	2.588	226	305.928	-
11	Biosimilar	100	10.573	113	1097.292	791.364
3	US-Neulasta	100	8.124	38	929.64	623.712
7	EU-Neulasta	100	7.57	67	834.516	528.588
12	Biosimilar	300	8.685	72	1008.468	702.54
4	US-Neulasta	300	7.978	72	971.772	665.844
8	EU-Neulasta	300	7.468	89	866.292	560.364
13	Biosimilar	1000	21.172	132	2258.928	1953
5	US-Neulasta	1000	22.719	118	2358.324	2052.396
9	EU-Neulasta	1000	19.574	113	1932.54	1626.612
14	Biosimilar	3000	48.573	125	4224.804	3918.876
6	US-Neulasta	3000	39.344	122	3404.232	3098.304
10	EU-Neulasta	3000	40.164	122	3566.724	3260.796

Note: Groups 3-14 were all pretreated with CPA and $AUEC_{0\text{-}t\ last}$ was calculated using the linear trapezoidal rule with ANC representing "plasma concentrations" collected between time zero (Day 1) and tlast (Day 12).
#: effective AUEC of the dose level groups 3 to 14: $AUEC_{Gr.\ 3\ to\ 14} - AUEC_{Gr\ 2}$; E_{max} = maximum response; t_{max} = time of E_{max}; AUEC = area under effect-time curve.

Of note, the Applicant also calculated the half maximum effective doses (ED_{50}) based on $AUEC_{eff}$ for each pegfilgrastim product to support a demonstration of biosimilarity, but with only 4 dose levels tested per product and the lack of a dose response between the 100 and 300 µg/kg dose levels, this analysis was not included in the review.

Other Observations
The following hematology parameters were reduced following CPA treatment, with no effects of the biosimilar, US- and EU-Neulasta groups on improving the depletion when compared to the CPA control group:

- Lymphocytes: began to recover starting at Day 5.
- Eosinophilic granulocytes: began to recover by Day 8.
- Platelets: began to recover by Day 6.

Of note, decreased RBC parameters were slower to recover in biosimilar, US- and EU-Neulasta-treated groups compared to the CPA control group.

Dosing Solution Analysis

The actual concentrations of the test item formulations ranged from 94.1% to 115.1% of the nominal pegfilgrastim concentrations. These values were within the acceptable range of 80% to 120% of the theoretical concentrations (i.e., ± 20%).

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Not Applicable.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No designated ADME studies were submitted.

5.2 TK

The toxicokinetics of MYL-1401H was evaluated in the comparative toxicology study TOX-071-001 and is summarized below in Section 6.2.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose comparative toxicology studies were submitted.

6.2 Repeat-Dose Toxicity

The batches of the biosimilar and EU-Neulasta used in the comparative toxicology study are the same as those used in the 3-way comparative PD study (Study No. PHA 071-001). Of note, these batches were not tested for G-CSF receptor binding or potency (in vitro proliferation assay in M-NFS-60 cells).

Study title: Comparative 28 Day Subcutaneous Repeat Dose Toxicity Study in Sprague Dawley Rats of a Proposed Biosimilar Pegylated Recombinant Human Granulocyte Colony-stimulating Factor (Peg-GCSF) and European Sourced Neulasta Followed by a 2 Week Treatment-free Recovery Period

Study no.:	TOX-071-001
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 6, 2013
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	Biosimilar ((b) (4)), Batch No. S13DBPEGI-0005, Purity: 98.1%; EU-Neulasta (9.8 mg/mL), Batch No. 1037627, Purity: 97.3%

Key Study Findings

- Similarity in pharmacodynamic, toxicity and toxicokinetic endpoints between the biosimilar and EU-Neulasta was demonstrated at one of the two comparative dose levels evaluated.

- Similarity at the 0.15 mg/kg dose level was not demonstrated for toxicity (limited histopathological evaluation) and toxicokinetics (possibly due to individual animal variability or dosing formulation differences).

Methods

Doses:	0, 0.15, 0.65, or 1.5 mg/kg/week biosimilar (Groups 1 to 4); 0.15 or 1.5 mg/kg/week EU-Neulasta (Groups 5 and 6, respectively)
Frequency of dosing:	Weekly
Route of administration:	SC
Dose volume:	5 mL/kg
Formulation/Vehicle:	Placebo for PEG-G-CSF injection
Species/Strain:	Hsd: Sprague Dawley
Number/Sex/Group:	Dosing Phase: 10/sex/group; Recovery Phase: 10/sex/group (controls and high dose only)
Age:	6 to 10 weeks at start of dosing
Weight:	♂ (males): 246.9 to 331.3 g, ♀ (females): 206.0 to 275.3 g
Satellite groups:	TK: 15/sex/group; Immunogenicity: 5/sex/group

Unique study design:	None
Deviation from study protocol:	None considered by the Study Director to affect the integrity of the study or the interpretation of results.

Observations and Times

Mortality:	Twice daily
Clinical signs:	Detailed examinations weekly
Body weights:	Pretest, weekly
Food consumption:	Weekly
Ophthalmoscopy:	Pretest and Week 4 (controls and high dose groups only)
Hematology, Clinical Chemistry, Coagulation:	♂+♀:Week 4 (♂ only: Week 5)
Urinalysis:	Week 4
Immunogenicity:	Predose, Week 4 and prior to necropsy. Immunogenicity analysis was considered unnecessary based on TK data and obtained samples were not analyzed.
Gross pathology:	At necropsy*
Organ weights:	At necropsy*
Histopathology:	At necropsy*
Toxicokinetics (samples taken from jugular vein):	Day 1 and Week 4: • 3 animals/sex/timepoint • Predose, 4, 8, 12, 24, 48, 72, 96, 120 and 144 hours (hr) postdose

* Necropsy was conducted on Day 30 of the dosing phase and Day 15 of the recovery phase. A full panel of tissues was examined microscopically for control and high dose dosing phase groups; only gross lesions were examined for other groups.

Results

Mortality

One 1.5 mg/kg EU-Neulasta-treated female was sacrificed moribund on Day 28. This female had clinical signs of blue color/swollen abdomen on Days 27/28 and sluggish behavior, rapid respiration, and raised hair and fur on Day 28. The cause of death was attributed to granulocytic leukemia. The relationship to EU-Neulasta cannot be excluded as this has never been observed in 28 day rat studies in the conducting laboratory.

Clinical Signs, Body Weights, Food Consumption, Ophthalmoscopy, Urinalysis

For these assessments, there were no remarkable test item-related findings or differences between the biosimilar or EU-Neulasta groups.

Hematology

Table 4 Comparative Hematology for the Biosimilar and EU-Neulasta

Dose (mg/kg/week)	0.15				1.5				0.65	
Test Item	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
Neutrophils (10^9/L)	fΔ	fΔ	fΔ	fΔ	fΔ	fΔ	fΔ	fΔ	fΔ	fΔ
Day 1 (postdose)										
4 hr	2↑	2↑	2↑	2↑	2↑	2↑	3↑	3↑	3↑	3↑
24 hr	7↑	9↑	6↑	8↑	7↑	7↑	13↑	16↑	11↑	11↑
48 hr	6↑	9↑	5↑	10↑	10↑	9↑	11↑	13↑	12↑	11↑
72 hr	2↑	3↑	2↑	2↑	11↑	12↑	18↑	17↑	12↑	13↑
Day 22 (postdose)										
4 hr	2↑	3↑	2↑	3↑	3↑	4↑	5↑	7↑	4↑	5↑
24 hr	17↑	16↑	26↑	17↑	16↑	13↑	26↑	57↑	22↑	47↑
48 hr	19↑	21↑	26↑	19↑	30↑	23↑	36↑	54↑	33↑	57↑
72 hr	6↑	7↑	9↑	4↑	20↑	20↑	38↑	58↑	15↑	34↑
Day 26	3↑***	3↑***	NE	NE	9↑***	8↑***	NE	NE	6↑***	NE
Day 30	20↑***	21↑***	30↑	1↑	21↑***	18↑***	31↑	51↑	26↑***	47↑
RBCs (10^{12}/L)	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc
Day 26	1↓	1↓	NE	NE	3↓	5↓	NE	NE	3↓	NE
Day 30	6↓**	6↓***	4↓	2↓	8↓***	12↓***	7↓	7↓	8↓***	9↓
Reticulocytes (10^9/L)	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc
Day 26	7↓	8↓	NE	NE	2↑	4↑	NE	NE	6↓	NE
Day 30	23↑**	30↑***	20↑	26↑	38↑***	34↑***	41↑	47↑	33↑***	52↑
Lymphocytes (10^9/L)	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc	%Δc
Day 26	3↑	3↓	NE	NE	45↑***	18↑	NE	NE	5↑	NE
Day 30	65↑***	82↑***	66↑	21↑	64↑***	60↑***	102↑	88↑	68***	71↑
Monocytes (10^9/L)	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc
Day 26	2↑***	2↑*	NE	NE	6↑***	5↑***	NE	NE	3↑***	NE
Day 30	5↑**	7↑***	7↑	1↑	7↑***	8↑***	10↑	10↑	6↑***	8↑
Eosinophils (10^9/L)	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc
Day 26	1↑	1↑	NE	NE	2↑***	2↑***	NE	NE	2↑*	NE
Day 30	2↑**	2↑***	3↑	1↑	2↑***	2↑**	3↑	4↑	2↑***	3↑***
Basophils (10^9/L)	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc
Day 26	2↑*	1↑	NE	NE	6↑***	5↑***	NE	NE	2↑***	NE
Day 30	10↑**	16↑***	10↑	1↑	21↑***	20↑***	24↑	24↑	14↑***	12↑

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Abbreviations: BIO = Biosimilar; EU-N = EU-Neulasta; RBC = red blood cells; fΔ = fold change relative to predose mean (Day 1 or Day 22); %Δc = percent change relative to control mean; fΔc = fold change relative to control mean; NE = not evaluated

- Despite differences in fold-change noted in Table 4, biosimilar and EU-Neulasta-treated females exhibited similar neutrophil counts on Day 22, 48 hours (48.75 vs 49.83 x 10^9 cells/L) and 72 hours (50.96 vs 53.54 x 10^9 cells/L) postdose and on Day 30 (42.14 vs 46.97 x 10^9 cells/L). Thus, differences can likely be attributed to differences in the predose values.

- Reductions in percent hematocrit, hemoglobin, and percent packed cell volume (PCV) and increases in mean corpuscular hemoglobin (MCH) and percent red cell distribution width (RDW) accompanied reductions in total RBCs.

Clinical Chemistry

Table 5 Comparative Changes in Clinical Chemistry for the Biosimilar and EU-Neulasta

Dose (mg/kg/week)	0.15				1.5				0.65	
Test Item	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
ALP (IU/L)	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc	fΔc
Day 26	4↑***	4↑***	NE	NE	11↑***	10↑***	NE	NE	7↑***	NE
Day 30	2↑***	2↑***	3↑***	1↑***	4↑***	4↑***	5↑***	6↑***	3↑***	4↑***

*** $p < 0.001$
Abbreviations: BIO = Biosimilar; EU-N = EU-Neulasta; ALP = alkaline phosphatase; fΔc = fold change relative to ALP control mean (♂: 112-123 IU/L; ♀: 71 IU/L); NE = not evaluated

- Transient statistically significant increases in calcium were observed in high dose biosimilar and EU-Neulasta-treated males (↑6% and ↑2%, respectively relative to controls).

- Increased AST levels were observed in males receiving 0.65 mg/kg biosimilar and in males receiving 0.15 or 1.5 mg/kg EU-Neulasta

Gross Pathology

Table 6 Comparative Changes in Gross Pathology for the Biosimilar and EU-Neulasta

Dose (mg/kg/week)	0.15				1.5				0.65	
Test Item	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
No. Animals Examined (Dosing/Recovery)	10/0	10/0	10/0	10/0	10/10	10/10	10/10	10/10	10/0	10/0
Large Spleen	7/-	8/-	4/-	1/-	9/3	9/4	9/3	8/0	9/-	5/-

Note: There were no large spleens observed in control animals, except for one male in recovery
BIO = Biosimilar; EU-N = EU-Neulasta; "-" = macroscopic observations were not made during recovery

Organ Weights

Table 7 Comparative Changes in Organ Weights for the Biosimilar and EU-Neulasta

Dose (mg/kg/week)	0.15				1.5				0.65	
Test Item	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
No. Animals Examined	10	10	10	10	10	10	10	10	10	10
Spleen %Δ										
Unadjusted	46↑	41↑	43↑	5↑	111↑	115↑	88↑	74↑	82↑	57↑
Organ to body weight ratio	45↑	43↑	36↑	7↑	116↑	116↑	79↑	70↑	78↑	56↑
Organ to brain weight ratio	48↑	42↑	40↑	2↑	112↑	115↑	84↑	68↑	81↑	54↑

BIO = Biosimilar; EU-N = EU-Neulasta; %Δ = percent change relative to control mean (♂: 0.835 g (unadjusted), 0.23% (organ to body weight), 44.26% (organ to brain weight); ♀: 0.643 g (unadjusted), 0.26% (organ to body weight), 37.09% (organ to brain weight). Of note, only dosing phase results are shown in the table, but spleen weight increases at 1.5 mg/kg trended toward reversibility during the recovery period.

Histopathology

Adequate Battery Yes

Peer Review No

Histological Findings

Table 8 Comparative Microscopic Findings for the Biosimilar and EU-Neulasta

Dose (mg/kg/week)	0		0.15				1.5				0.65	
Test Item	Control		BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♀	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
Finding												
Femur and marrow												
No. Animals Examined	10/0	10/0	0/0	1/0	0/0	0/0	10/0	10/0	10/0	9/0	0/0	0/0
Increased granulopoiesis												
Moderate	0/-	0/-	-/-	1/-	-/-	-/-	10/-	10/-	10/-	9/-	-/-	-/-
Sternum and marrow												
Increased granulopoiesis												
Moderate	0/-	0/-	-/-	-/-	-/-	-/-	10/-	10/-	10/-	9/-	-/-	-/-
Spleen												
No. Animals Examined	10/2	10/0	8/0	8/0	4/0	1/0	10/3	10/4	10/3	9/0	9/0	5/0
Hematopoiesis												
Minimal	5/1	2/-	0/-	0/-	0/-	0/-	0/0	0/1	0/0	0/-	0/-	0/-
Slight	4/0	1/-	1/-	0/-	0/-	0/-	0/3	0/3	0/0	0/-	0/-	0/-
Moderate	0/0	0/-	7/-	8/-	4/-	1/-	10/0	10/0	10/2	9/-	9/-	5/-

Dose (mg/kg/week)	0		0.15				1.5				0.65	
Test Item	Control		BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♀	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
Liver												
No. Animals Examined	10/3	10/1	0/0	4/0	3/0	4/0	10/3	10/4	10/0	9/2	4/0	1/0
Hematopoiesis												
Minimal	1/1	0/0	-/-	0/-	1/-	1/-	0/3	0/1	0/-	0/1	1/-	0/-
Slight	0/0	0/0	-/-	4/-	2/-	2/-	10/0	10/2	10/-	9/-	3/-	1/-

BIO = Biosimilar; EU-N = EU-Neulasta; "-" = group was not evaluated; No. Animals Examined (Dosing Phase/Recovery)

- Also observed was slight myocarditis of the heart in 1/10 1.5 mg/kg biosimilar-treated males, minimal inflammatory cell foci in the pancreas each in 2/10 1.5 mg/kg biosimilar- and EU-Neulasta-treated males, minimal adrenal cortical vacuolation in 1/10 1.5 mg/kg biosimilar-treated males and 2/10 1.5 mg/kg EU-Neulasta-treated males, and minimal adrenal zona glomerulosa vacuolation in 2/10 1.5 mg/kg biosimilar-treated females.

- Minimal fasciitis/fibrosis and occasional hemorrhage were also recorded in animals given the biosimilar or EU-Neulasta and in control animals.

Toxicokinetics

- Similar levels of exposure (C_{max} and AUC) were observed between the biosimilar and EU-Neulasta at the 1.5. mg/kg dose level, but not at the 0.15 mg/kg dose level. The Applicant noted there was large animal variability. It is also noted that the dosing formulation concentrations for the 0.15 mg/kg biosimilar dose were >20% lower than the intended concentration at this dose level, which may have also contributed to lower exposures.

- Other similarities in TK parameters between the biosimilar and EU-Neulasta:

    - Concentrations were below the limit of quantification between 96 and 144 hours, with elimination being slower with increasing dose level.

    - Exposure increases were greater than dose proportional.

    - Males had lower exposures than females.

    - Maximum plasma concentrations were reached within 12 to 24 hours (t_{max}).

    - The half-life ($t_{1/2}$) was between 4 and 10 hours.

    - Accumulation ratios were less than 2.

Table 9 Comparison of TK Parameters for the Biosimilar and EU-Neulasta

Dose (mg/kg/week)	0.15				1.5				0.65	
Test Item	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	EU-N	BIO	
Sex	♂	♂	♀	♀	♂	♂	♀	♀	♂	♀
C_{max} (ng/mL)										
D1	86.9	140	258	268	2340	2280	4200	4030	1010	1590
D22	98.9	269	315	437	2410	2500	4260	2900	565	1380
t_{max} (hr)										
D1	12	12	12	24	12	24	12	12	24	12
D22	12	12	12	12	24	12	12	24	24	12
$t_{1/2}$ (hr)										
D1	6.98	6.2	4.93	NR	4.74	NR	4.73	4.13	4.91	4.83
D22	NR	8.29	NR	4.99	8.31	NR	6	6.18	10.1	5.32
$AUC_{(0-t)}$ (ng.hr/mL)										
D1	1700	2640	5190	6020	113000	106000	170000	158000	33000	48600
D22	1570	4080	4860	6860	83600	79500	148000	124000	13200	38600
C_{max}/Dose										
D1	580	931	1720	1780	1560	1520	2800	2690	1550	2450
D22	660	1800	2100	2920	1600	1670	2840	1930	870	2120
$AUC_{(0-t)}$ /Dose										
D1	11300	17600	34600	40100	75200	70500	113000	106000	50800	74700
D22	10500	27200	32400	45700	55700	53000	98600	82400	20300	59300
AR_{Cmax}										
D22	1.14	1.93	1.22	1.63	1.03	1.1	1.01	0.719	0.562	0.865
$AR_{AUC(0-t)}$										
D22	0.922	1.55	0.938	1.14	0.741	0.752	0.869	0.78	0.399	0.794
C_{max} Bio ratio*										
D1		0.623		0.964		1.03		1.04		
D22		0.367		0.721		0.963		1.47		
$AUC_{(0-t)}$ Bio ratio*										
D1		0.645		0.862		1.07		1.07		
D22		0.384		0.709		1.05		1.2		

BIO = Biosimilar; EU-N = EU-Neulasta; C_{max} = maximum plasma concentration; t_{max} = time of maximum concentration from the concentration/time profile; $t_{1/2}$ = half-life; AUC_{0-t} = area under the concentration-time curve from hour 0 to the last measurable concentration, estimated by the log/linear trapezoidal rule; AR = accumulation ratio; * Biosimilarity ratio (Biosimilar/EU-Neulasta); D = day; NR = no result calculable

Dosing Solution Analysis

One out of 3 control samples failed to meet criteria. It was noted that there was insufficient sample for repeat analysis. Six out of 15 samples were outside the ± 20% target detailed in the protocol (highlighted in Table 10), including all 3 for the 0.15 mg/kg

biosimilar doing group (Group 2). The Applicant noted that there was no opportunity to reformulate so these results were accepted and taken into account during data interpretation.

Table 10 Formulation Sample Results in the Comparative Toxicology Study

(Excerpted from the study report)

Dose Group	Occasion	Back Calculated Conc (pg/mL)	Back Calculated Conc (mg/mL)	Dosed Conc (mg/mL)
1	19th Nov	X1	X1	0.000
1	9th Dec	<300.00	<LLOQ	0.000
1	11th Dec	<300.00	<LLOQ	0.000
2	19th Nov	22662943.26	0.023	0.030
2	9th Dec	19694145.95	0.020	0.030
2	11th Dec	23402999.59	0.023	0.030
3	19th Nov	147132795.56	0.147	0.130
3	9th Dec	100060418.05	0.100	0.130
3	11th Dec	101093373.27	0.101	0.130
4	19th Nov	242703950.67	0.243	0.300
4	11th Dec	245298192.61	0.245	0.300
4	11th Dec	279122732.57	0.279	0.300
5	19th Nov	24022778.77	0.024	0.030
5	9th Dec	34704324.13	0.035	0.030
5	11th Dec	27296732.54	0.027	0.030
6	19th Nov	271789189.23	0.272	0.300
6	9th Dec	212085217.45	0.212	0.300
6	11th Dec	257747707.62	0.258	0.300

7 Genetic Toxicology

No genetic toxicology studies were submitted or are required since pegfilgrastim is a biologic [ICH Guidance S6(R1)].

8 Carcinogenicity

No carcinogenicity studies were submitted or are required according to the principles outlined in ICH Guidances S9 and S6(R1).

9 Reproductive and Developmental Toxicology

No new studies were submitted. Data from reproductive and developmental toxicology studies conducted with pegfilgrastim and already incorporated into the US-Neulasta label will be applied to the FULPHILA label with language updated to comply with PLLR.

10 Special Toxicology Studies

None were submitted.

11 Integrated Summary and Safety Evaluation

See Executive Summary.

12 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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09/05/2017

CHRISTOPHER M SHETH
09/05/2017